

FINAL REPORT

THE KEY INVOLVEMENT OF POLY(ADP-RIBOSYL)ATION IN DEFENSE AGAINST TOXIC AGENTS: MOLECULAR BIOLOGY STUDIES (AFOSR grant FA9550-04-1-0395)

January 2005 to May 2007

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B. List of manuscripts submitted/published during the grant support (2005-2007).

PUBLISHED

- Espinoza LA, **Smulson ME**, Chen, Z. Prolonged poly(ADP-ribose) polymerase-1 activity regulates JP-8-induced sustained cytokines expression in alveolar macrophages. *Free Radic. Biol. Med.* 42:1430-1440 (2007).
- Amiri KI, Ha HC, **Smulson ME**, Richmond A. Differential regulation of CXC ligand 1 transcription in melanoma cell lines by poly(ADP-ribose) polymerase-1. *Oncogene*. 2006 25:7714-7722 (2006).
- Espinoza LA, Tenzin F, Cecchi AO, Witten ML, **Smulson ME**. Expression of JP-8-induced inflammatory genes in AEII cells is mediated by NF- κ B and PARP-1. *Am. J. Respir. Cell Mol. Biol.* 35:479-487 (2006).
- Espinoza LA, Valikhani M, Cossio MJ, Carr T, Jung M, Hyde J, Witten ML and **Smulson ME**. Altered expression of g-synuclein and detoxification-related genes in lungs of rats exposed to JP-8. *Am. J. Respir. Cell Mol. Biol.* 32, 192-200 (2005).
- Chiba-Falek O, Kowalak JA, **Smulson ME**, Nussbaum R. Regulation of α -synuclein expression by Poly (ADP ribose) polymerase-1 (PARP-1) binding to the NACP-Repl polymorphic site upstream of the SNCA gene. *Am. J. Hum Genet* 76,478-492 (2005).
- Chasovskikh S, Dimtchev A, **Smulson ME**, Dritschilo A. DNA transitions induced by PARP binding to cruciform structures in supercoiled plasmids *Cytometry: Part A*. 68:21-27 (2005).

INVITED CHAPTERS

Boulares H, Yakovlev AG and Smulson ME PARP-1 mediated regulation of apoptotic endonuclease DNAS1L3. In: Bürkle A [ed.] Poly(ADP-Ribosylation). Molecular Biology Intelligence Unit. Landes Bioscience, Georgetown, TX, USA. *In press*. (2005).

C. Scientific personnel supported by this grant

Mark E. Smulson, Ph.D., 25%
Luis Espinoza, Ph.D 100%
Fnu Tenzin MS 50%
Zun Chen MD 100%

D. Inventions/Patents/Discoveries

N/A

E Collaborators

A portion of the data published has been cooperation and formal collaboration with Dr. Mark Witten (co-author on two during the period 2005-2006).

I wrote a letter of collaboration for Dr. Frank Witzman's AFOSR Renewal application (06/05/2005).

Among the most affected alveolar cells are alveolar type II epithelial (AII) and pulmonary alveolar macrophages (PAM). These are characterized by a fuel induced lung inflammatory condition (parenthetically, roles for PARP in the inflammatory process has recently been described). Additionally, a recent study demonstrated that AII cells have the ability to secrete cytokines, which are a critical inflammatory mechanism. Witten in his Renewal is currently focusing on the above topics as well as the role which he has already shown that Substance P analogue, Sar9, Met (O2)11-substance P, treatment is effective in attenuating the JP-8 jet fuel-induced lung injury.

F. Honors or Awards received by you or your personnel while being supported by AFOSR over the PAST YEAR.

N/A.

G. Key Findings/Results/Accomplishment

[Note: for much of 2007 (7 months), the PI was hospitalized after a ten hour operation. Accordingly, the PI could not submit a new (renewal) application on this long term, -20years -Air Force supported work on PARP and toxicology'

With renewed health- and insights, the PI hopes, in the future, to re explore , with the A.F. this increasingly interesting topic.]

Our study during the period 2005-2007 focused in lung cell as critical in the regulation of airway inflammation in response to environmental pollutants. We have found that a prolonged exposure of alveolar macrophages to a nonlethal dose (8 µg/ml) of JP-8 induced the persistent expression of several pro-inflammatory factors in macrophages (IL-1, iNOS, and COX-2) (**Free Radic. Biol. Med.** 42,1430-1440, 2007), as well as cell adhesion molecules (ICAM-1 and VCAM-1) and in respiratory epithelial cells (TNF-α, IL-6, and IL-8) (**Am. J. Respir. Cell Mol. Biol.** 35,479-487, 2006). Because poly(ADP-ribose) polymerase (PARP-1), a coactivator of NF-κB, regulates inflammatory responses and associated disorders in the airways, we determined whether JP-8 induces the poly(ADP-ribosylation) automodification of PARP-1 in alveolar macrophages. We observed that PARP-1 is activated in a time-dependent manner in both types of cells, which was temporally coincident with the prolonged activation of NF-κB and with the augmented expression of the pro-inflammatory factors described above. A lower dilution of JP-8 (4 µg/ml) also increased the activity of PARP-1 as well as the expression of iNOS and COX-2 in macrophages. These findings indicate that lower doses of JP-8 also affect the regulation of proinflammatory factors in pulmonary cells. Together, these results demonstrate that an

extensive induction of PARP-1 might coordinate the persistent expression of proinflammatory mediators in alveolar macrophages and epithelial cells activated by aromatic hydrocarbons. These results evidenced that a persistent exposure of airway cells to aromatic hydrocarbons may have deleterious effects on pulmonary function.

The key observations of our studies on the Affymetrix Chip Microarray analysis of lung mRNA isolated for mice exposed to JP-8 was published (*Am. J. Respir. Cell Mol. Biol.* 32,192-200 (2005)). We used microarray analysis to characterize changes in the gene expression profile of lung tissue induced by exposure of rats to an aerosol of JP-8 at a concentration of 171 or 352 mg/m³ for 1 h per day for 7 days, with the higher dose estimated to mimic the level of occupational exposure in humans. The expression of 56 genes was significantly affected by a factor of ≤ 0.6 or ≥ 1.5 by JP-8 at the low dose, with the expression of 86% of these genes being down-regulated by JP-8. The expression of 66 genes was similarly affected by JP-8 at the higher dose, with the expression of 42% of these genes being up-regulated by the fuel. Prominent among the latter genes was that for the **centrosome-associated protein γ -synuclein**, whose expression was increased 5.5-fold by JP-8. The expression of various genes related to antioxidant responses and detoxification, including those for glutathione S-transferases and cytochrome P450 proteins, was also up-regulated by JP-8 at the higher dose. The microarray data were confirmed by quantitative reverse transcription and polymerase chain reaction analysis. Our extensive data set may thus provide important insight into the pulmonary response to occupational exposure to JP-8 in humans.

H. Transitions/Technology Transfers: none

None this year